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Monthly Focus: Endocrine & Metabolic

Glycogen phosphorylase inhibitors for treatment of type 2 diabetes mellitus

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Type 2 diabetes mellitus is a severe disease with large economic consequences, which is significantly under-diagnosed and incompletely treated in the general population. Control of blood glucose levels is a key objective in treating diabetic patients, who are most often prescribed one or more oral hypoglycaemic agents in addition to diet and exercise modification as well as insulin. In spite of the availability of different classes of hypoglycaemic drugs, treatment regimens are often unable to achieve an intensive degree of glucose control known to most effectively reduce the incidence and severity of diabetic complications. Hepatic glucose output is elevated in type 2 diabetic patients and current evidence indicates that glycogenolysis (release of monomeric glucose from the glycogen polymer storage form) is an important contributor to the abnormally high production of glucose by the liver. Glycogen phosphorylase is the enzyme that catalyses this release and recent advances in new inhibitors of this structurally and kinetically well studied enzyme have enabled work which further delineate the pharmacological and physiological consequences of inhibiting glucose production by this pathway. Most notably, these agents lower glucose in diabetic animal models, both acutely and chronically, appear to affect both gluconeogenic and glycogenolytic pathways and demonstrate potential for a beneficial effect on cardiovascular risk factors. Cumulatively, this information has bolstered interest and promise in glycogen phosphorylase inhibitors (GPIs) as potential new hypoglycaemic agents for treatment of type 2 diabetes mellitus.

Keywords: *antihyperglycaemic, blood glucose, diabetic animal models, diabetic complications, drug discovery, enzyme, gluconeogenesis, glycogen phosphorylase inhibitor, glycogenolysis, hepatic glucose production, indole inhibitor site, myocardial infarction, obesity, oral hypoglycaemic agents, stroke, type 2 diabetes mellitus therapy*

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1. Introduction

Diabetes mellitus is a complex disease process, which is characterised by altered glucose metabolism and insulin utilisation. Estimates indicate that diabetes shortens life expectancy by an average of 15 years. Since 1980, the age-related death rate for diabetes has increased 30%, in contrast to other serious diseases, such as stroke, cardiovascular disease and cancer where mortality rates have levelled off or declined [1-5]. Management of diabetes requires a diligent approach of understanding and support on the part of the patient, family members and the many practitioners who treat this

Figure 1: Diabetes worldwide: increasing prevalence from 4.0 - 5.4% of adults over 20 years of age. Source: [8,12].

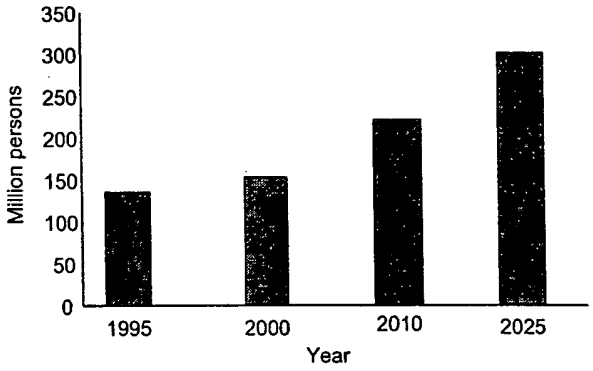


Table 1: Incidence of cardiovascular events during a seven-year follow-up in relationship to history of myocardial infarction [14].

Event	Non-diabetics		Type 2 diabetics	
	Prior MI	No history of MI	Prior MI	No history of MI
Fatal/non-fatal MI at follow-up	18.8	3.5	45	20.2
Fatal/non-fatal stroke at follow-up	7.2	1.9	19.5	10.3

MI: Myocardial infarction.

progressive disease. Comprehensive guidelines, which are continually re-assessed and revised, underscore the dramatic and dynamic medical need represented by this disease [5,6]. Approximately 16 million people in the US have type 2 diabetes mellitus, which represents about 90 - 95% of all US diabetes cases [7]. The prevalence of diabetes in adults over 20 years of age is now increasing by 5.4% per year. According to the American Diabetes Association, there are about 800,000 newly diagnosed diabetics per year in the United States alone [8,9]. The prevalence of diabetes is projected to increase to over 25 million by 2010 in the US and to over 300 million by 2025 worldwide (see **Figure 1**) [10-13]. This dramatic increase in diabetes prevalence is associated with general ageing of the world population and increased industrialisation of many countries, particularly in Asia and Africa. In addition, three specific parameters,

- Obesity
- Lack of physical activity
- Genetic predisposition

are driving the increased incidence of the disease in susceptible individuals. To this end, public health strategies directed at surveillance, diagnosis and comprehensive management are critical in dealing with the clinical and health economic burden of diabetes [12,13]. The cornerstone of diabetes management lies in glycaemic control but effective treatment clearly also requires education, lifestyle modification and aggressive management of blood pressure, serum lipids, smoking cessation and other measures directed at reducing the burden of associated cardiovascular disease. The significance of macrovascular complications of diabetes (myocardial infarction, peripheral vascular disease and stroke), particularly type 2 diabetes, is strikingly evident in the epidemiological findings of Haffner and colleagues in a 1998 report [14]. In a seven-year prospective analysis of patients with or without a history of myocardial infarction and with or without a diagnosis of type 2 diabetes, it was determined that type 2 diabetes was a better predictor for having a heart attack than was a previous heart attack itself (**Table 1**).

Results from the Diabetes Control and Complications Trial set a standard for aggressive glycaemic control in patients with type 1 disease. More recently, positive results from the United Kingdom Prospective Diabetes Study (UKPDS) demonstrated a 25% decrease in microvascular complications in aggressively treated patients with type 2 diabetes. In the UKPDS, a 1% reduction in glycosylated haemoglobin (HbA1c) level correlated with a 7% reduction in overall mortality and an 18% reduction in combined fatal and non-fatal myocardial infarction [15]. Further analyses from the UKPDS also support the combined approach of aggressive glycaemic control plus blood

pressure control to reduce the clinical and economic burden of the micro- and macro-vascular complications associated with diabetes [5]. It is important to note, however, that considerable challenges remain in defining adequate standards of control and in identifying effective treatment strategies that will fully impact disease progression and specific cardiovascular end points [16,17]. Adequate glycaemic control in diabetes treatment is defined as achieving an HbA1c level of 7%. Health status and clinical outcomes support this objective, as it has been demonstrated that medical care charges increase significantly for every 1% increase in HbA1c above this 7% level. The results from the most recent National Health and Nutrition Evaluation Study (NHANES), however, suggest that only 4 out of 10 patients treated for diabetes meet this treatment target. In order to accomplish better management of glycaemia, clinicians may move from initial treatment with one agent to more aggressive intervention with multiple oral therapies, as well as insulin.

Oral hypoglycaemic agents have various mechanisms of action. Sulphonylureas, a leading class of antidiabetic agents dominated by generic compounds, are insulin secretagogues used to treat 3.5 million US patients. Another 2 - 4 million patients are treated with metformin (Glucophage™), which lowers glucose levels by multiple mechanisms. Glitazones are the most recently introduced class of antidiabetic agents and work by alleviating insulin resistance in the periphery. Troglitazone (Rezulin™) was prescribed to some 1 million patients in its first year but was withdrawn in 2000 from the US market, due to liver safety concerns. Pioglitazone (Actos™) and rosiglitazone (Avandia™) are follow-ons in this class. Given as monotherapy, the agents listed above are not uniformly effective in achieving complete glycaemic control and are limited by secondary failure or undesired side effects. Diabetics progressing to insulin currently incur severe hypoglycaemic risk, weight gain and daily parenteral dosing. A clear medical need exists, therefore, for oral antidiabetic agents that produce better glycaemic control as monotherapy or in combination with existing agents. In this article, we discuss the potential for developing a new class of oral hypoglycaemic agents for antidiabetic therapy that work through a mechanism of inhibiting the action of the enzyme glycogen phosphorylase action; we review the research progress which has been made towards discovering orally-active glycogen phosphorylase inhibitors.

2. Role of glycogen phosphorylase in diabetes

2.1 Aetiology of type 2 diabetes

Although the exact causes of type 2 diabetes have not yet been identified, it is well established that diabetes is a polygenic disease, characterised by multiple defects in insulin action in muscle, adipose tissue, liver and defects in pancreatic insulin secretion. The relative importance of these distinct events in the aetiology of diabetic hyperglycaemia is not clear [18]. Over the last decade, peripheral insulin resistance in skeletal muscle and adipose tissues has been viewed as the primary catalyst for inducing glucose abnormalities and frank diabetes. However, recent work elucidating the molecular pathways of insulin action and glucose homeostasis suggest that functional abnormalities in the pancreatic -cells and/or the regulation of hepatic glucose production may be the first events contributing to the development of a diabetic phenotype [19].

2.2 Role of hepatic glucose production and the glycogenolysis component in type 2 diabetes

Hepatic glucose production is an important target for diabetic therapy because the liver is the major regulator of plasma glucose levels in the post-absorptive (fasted) state and also because the rate of hepatic glucose production[1] in diabetic patients is significantly elevated relative to healthy subjects [18,20-22]. Likewise, in the postprandial (fed) state, where the liver has a proportionally smaller role in supplying glucose to the circulation, hepatic glucose production is still abnormally high in diabetic patients [23,24]. Hepatic glucose production is finely regulated by the counteractive effects of glucagon and insulin, among other factors but in the diabetic state, where lack of insulin (type 1 diabetes) or resistance to insulin action (type 2 diabetes) is coupled with hyperglucagonaemia, liver glucose production is abnormally high and contributes to the hyperglycaemia observed.

[1]In this review the term 'hepatic glucose production' will be used instead of 'endogenous glucose production' since this discussion focuses on the liver. The reader should be aware the kidney provides a minor component (ca. 10%) of endogenous glucose production.

Equation 1:

Hepatic glucose production = Glycogenolysis + Gluconeogenesis

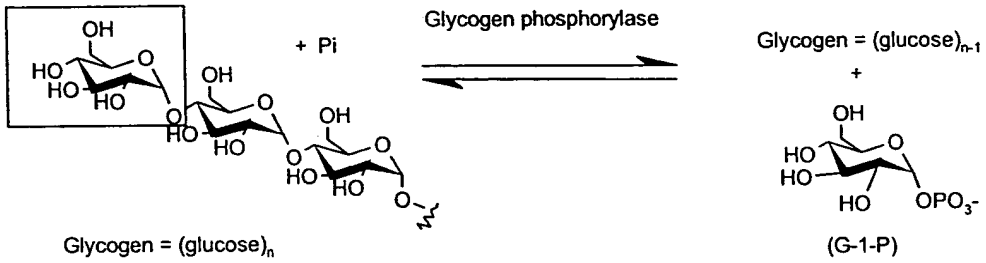
Hepatic glucose production is the net sum of two metabolic processes performed by the liver: glycogenolysis (breakdown of the glucose polymer glycogen) and gluconeogenesis (synthesis of glucose from 3-carbon precursors). The measurement of hepatic glucose production in post-absorptive subjects is commonly performed by monitoring changes in the specific activity of an infused radioactive tracer, such as [6-^{3}H] glucose [25]. However, *in vivo* measurements to determine the contributions of glycogenolysis *versus* gluconeogenesis to the rate of hepatic glucose production are encumbered with challenges to existing methodology that make quantitative assessment difficult. It is not methodologically feasible to measure both hepatic glycogenolysis and gluconeogenesis simultaneously in a human subject; therefore the standard experimental practice is to measure the rate of hepatic glucose production plus one of the other variables (glycogenolysis or gluconeogenesis), then solve for the remaining variable according to **Equation 1**.

Some of the discrepancies reported in the literature concerning the contribution of gluconeogenesis and glycogenolysis to hepatic glucose production may originate from differences in the experimental methods used to quantitate these processes. Methods for measuring gluconeogenesis include splanchnic arterio-venous (A-V) substrate balance, radioisotope dilution, various mass isotopomer dilution analyses (MIDA) and deuterium enrichment of *de novo* synthesised glucose. Some of the methods described for measuring glycogenolysis are serial liver biopsy for biochemical determination of glycogen concentration and NMR spectroscopy to assess changes in liver ^{13}C-glycogen content. Invasive techniques, such as liver biopsy and splanchnic balance (A-V difference) are generally not performed in clinical research. The methods available for measuring gluconeogenesis are limited by a number of factors, including isotope exchange and dilution, *in vivo* sampling constraints, substrate cycling phenomena, assumptions and estimated constant values used in the equations for calculating results and the contribution of renal gluconeogenesis to the glucose production rate [26]. The MIDA technique based on measuring ^{13}C-glycerol flux for determining gluconeogenesis has come under critical review [27-29], while other stable isotope-based techniques appear to be gaining acceptance [30,31]. The measurement of hepatic glycogenolysis by NMR spectroscopy has been favourably reviewed [32] but the results obtained may be influenced by the dietary pretreatment regimen required to achieve hepatic glycogen ^{13}C enrichment, assumptions regarding liver volume and variations in subject positioning in the magnet [33,34].

Using the methods described above, estimates vary on the relative contribution of gluconeogenesis and glycogenolysis to hepatic glucose production. In healthy, post-absorptive subjects, the proportion of hepatic glucose production reported to be attributed to gluconeogenesis ranges from 40 - 70% (with glycogenolysis comprising the balance, e.g., 30 - 60%). With more prolonged fasting (> 16 h), gluconeogenesis appears to be the predominant (> 90%) contributor to hepatic glucose production [35-37]. These estimates may be complicated by hepatic glucose-glycogen cycling [24,38,39] which is not taken into consideration in some of the methods described above. It is difficult to conclude firmly that either parameter has a quantitatively greater role to the regulation of hepatic glucose production in healthy volunteers; indeed, many conclude that the contributions are similar [33]. In type 2 diabetics, the situation is also uncertain, since in the few studies performed, the gluconeogenic contribution to the observed rate of hepatic glucose production has ranged from 25 - 88%. It is generally recognised that type 2 diabetics display elevated hepatic glucose production; in some studies this is due to proportionally greater gluconeogenic rates [34,36,37] but others [40,41] suggest that glycogenolysis is the elevated component.

It is unclear, therefore, whether inhibition of hepatic glycogenolysis can normalise hepatic glucose production in type 2 diabetic patients. There does, however, appear to be a prospect of affecting 30 - 60% of total hepatic glucose production by blocking glycogenolysis, or up to ~ 80% of total hepatic glucose production if blockade of glucose-glycogen cycling also occurs [38,39]. Finally, episodic hypoglycaemia is observed in patients with type VI glycogen storage disease who lack hepatic glycogen phosphorylase [42]; this supports the contention that pharmacological interruption of hepatic glycogenolysis could reduce glucose levels in type 2 diabetics.

Figure 2: The enzyme glycogen phosphorylase associates with the glycogen macromolecule and catalyses the addition of phosphoric acid across the 1,4-anomeric linkage, releasing glucose-1-phosphate and a glycogen macromolecule shortened by one sugar residue.



3. Glycogen phosphorylase and inhibitors

3.1 Occurrence and properties of glycogen phosphorylase

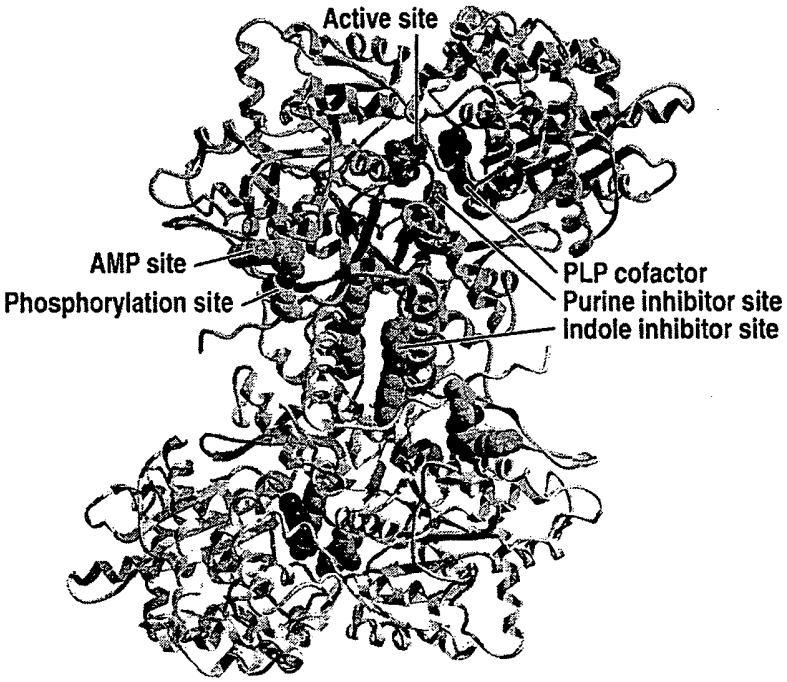
Under physiological conditions, the enzyme glycogen phosphorylase associates with the glycogen macromolecule and catalyses the addition of phosphoric acid across the 1,4-anomeric linkage, releasing glucose-1-phosphate and a glycogen macromolecule shortened by one sugar residue (**Figure 2**). Humans, as well as other non-human mammalian species, express three distinct isoforms of this enzyme, liver, brain and skeletal muscle. These proteins are products of distinct genes, located on (human) chromosomes 14, 20 and 11, respectively. All three human isoforms of glycogen phosphorylase have a molecular weight of ~ 97 kDa (for the monomeric form) and share approximately 80% amino acid identity [43]. The active form of the enzyme is a homodimer consisting of two identical monomeric subunits (194 kDa total), associated with very high affinity to one another. Phosphorylase activity is regulated physiologically by hormonal and neuronal signals that alter phosphorylation at serine 14 in each subunit. In the liver it is the phosphorylated form (GP*a*) that has the preponderance of glycogenolytic activity. Catalytic activity of the unphosphorylated form of liver phosphorylase (GP*b*) is negligible by comparison.

3.1.1 Identity and function of ligand binding sites of glycogen phosphorylase

The liver isoform of human glycogen phosphorylase (HLGP) is the target for therapeutic intervention with glycogen phosphorylase inhibitors (GPIs) in type 2 diabetes. HLGP is a principal determinant of hepatic glucose production from glycogenolysis and blood glucose levels in type 2 diabetics. In contrast, the main role of the human muscle isoform of phosphorylase (HMGP) is to initiate metabolism of muscle glycogen to provide energy to the muscle during exercise, so the muscle isoform is not the principal target of a GPI intended to treat hyperglycaemia. In addition to phosphorylation at serine-14, phosphorylase activity is also regulated physiologically by glucose, ATP and glucose-6-phosphate, which act as inhibitors and by AMP, which is an activator. X-ray crystallography studies, primarily using the rabbit muscle and human liver enzymes, have established binding sites for these ligands and for other GPIs. The physical relationship of these sites to the phosphorylation site and pyridoxyl phosphate co-factor is shown in **Figure 3**. Glucose binds at the active site where the phosphorylase reaction occurs. AMP, ATP and G-6-P bind at a distant allosteric site termed the nucleotide activation site or AMP site. The glycogen binding site associates GP with the glycogen particle. Additionally, two other binding sites have been identified in mammalian GPs for which no physiological role has yet been firmly established. Inhibitory ligands for these sites will be discussed below. The first is the purine or nucleoside inhibitor site, which binds caffeine and other nucleosides and is located about 10Å from the catalytic site. The second is the indole inhibitor site, which exists within a solvent-filled cavity at the subunit interface of the homodimeric enzyme and binds certain indole-containing inhibitors (**Figure 3**). As discussed below, this binding site has only recently been reported and has no known physiological ligand.

Various species and tissue isoforms of GP, either in the phosphorylated or unphosphorylated state, have been used to assess the inhibitory activity of putative inhibitors. Most work has used phosphorylated or unphosphorylated rabbit muscle glycogen phosphorylase (RMGP*a* and RMGP*b*), or the phosphorylated recombinant human liver enzyme (rHLGP*a*). Certain inhibitors have been shown to possess qualitatively similar enzyme inhibitory activity independent of

Figure 3: Composite representation of glycogen phosphorylase homodimer and its effector sites. The figure is a composite created in ribbons [83] of the co-ordinates of HLGP*a* complexed with AMP [84] and ligands from the crystal structures of HLGP*a* complexed with CP-403,700 and the glucose analogue, 1-GlcNac (2) [68] and the complex of HLGP*a*/CP-403700/1-GlcNac/caffeine (Ekstrom, JE, Rath VL, unpublished data).



phosphorylation state (e.g., RMGP*a* and RMGP*b*), or tissue isoform (e.g., rHLGP*a* and recombinant human muscle GP*a*, rHMGP*a*) in some of the articles cited herein. However, structural differences exist between enzyme isoforms and the experimentally determined inhibitor activity may depend on phosphorylation state and concentration of allosteric effectors (e.g., AMP or glucose). Consequently caution must be exercised in comparing enzyme inhibitory potencies measured under non-identical conditions.

3.2 Synthetic inhibitors of glycogen phosphorylase

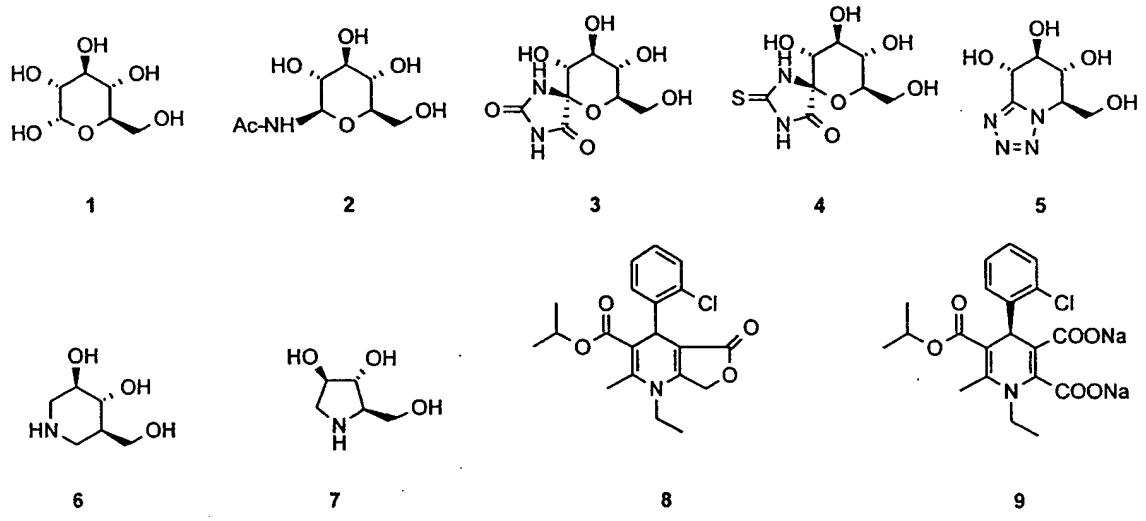
3.2.1 Active site inhibitors

-D-Glucose (**1**) inhibits RMGP*b* with a K_i of 2 mM by binding at the glucosyl recognition site for its substrate glycogen and product glucose-1-phosphate, respectively (G-1-P is also a substrate for the reverse reaction) [44]. While this site shows high selectivity for most elements of the glucosyl structure [45], latitude for structural changes at C-1 is greatest and synthetic glucose analogue inhibitors of increased affinity have been achieved and shown to bind to this site [46]. The most potent monocyclic glucose analogue inhibitor is N-acetyl- -D-glucosamine (**2**, [47]), a 32 M inhibitor (K_i) of RMGP*b* and RMGP*a*. Glucose analogues showing the highest inhibitory activity are bicyclic compounds wherein a second ring is fused to the C-1. Glucopyranose spirohydantoin **3**, with a K_i of 3.0 M *versus* RMGP*b*, is the most potent glycogen phosphorylase inhibitor which has been shown to bind at the active site [48]. The sulphur analogue **4** also possesses similar enzyme inhibitory activity to **3** for phosphorylated and unphosphorylated rabbit muscle and rat liver phosphorylases [49]. Tetrazole **5** is also an inhibitor of RMGP*b* (K_i = 58 mM) which binds to the active site. Inorganic phosphate is hydrogen-bonded both to **5** and to the phosphate of the co-factor pyridoxal-6-P in a ternary complex resembling the putative glycogenolysis transition state complex [50].

3.2.2 Azasugars

Extensive literature exists on inhibition of glycosidases with naturally occurring polyhydroxylated nitrogen-containing heterocycles [51]. At least two of these heterocycles are also potent inhibitors of glycogen phosphorylase, (*R,R,R*)-3-hydroxymethyl

Figure 4: Chemical structures of glucose and other glycogen phosphorylase inhibitors.



4,5-piperidinediol, **6** (isofagomine) [52,53] and (*R,R,R*)-2-hydroxymethyl-3,4-pyrrolidinediol, **7** (1,4-dideoxy-1,4-imino-D-arabinitol, DAB) [54]. These structures have some features in common with glucose (**1**) but crystallographic determination of the binding site of either **6** or **7** is not reported. Several patent applications have been filed directed toward **6** and **7** and analogues for treating diabetes by inhibiting glycogen phosphorylase and liver glucose production [101-104].

3.2.3 AMP site inhibitors

Compound **8** (BAYR3401) is reported to inhibit hepatic glycogenolysis *in vivo* [55] following its metabolic conversion to compound **9** (BAY W1807) [56]. Compound **9** is a 1.6 nM inhibitor (K_i) of the unphosphorylated rabbit muscle enzyme and binds at the AMP-stimulatory site [57]. While **9** inhibits the phosphorylated rabbit muscle enzyme much less effectively (K_i of 19 M in the presence of AMP), it does so by making very similar binding interactions at this site [58]. In isolated hepatocytes and perfused liver from rats, the inactivation and inhibition of phosphorylase is attributed to the interaction of **9** with GP*a* (not GP*b*). Data showing hypoglycaemic activity for several lactone analogues of **8**, as well as for compound **9** and its cyclic anhydride, are reported [105-107].

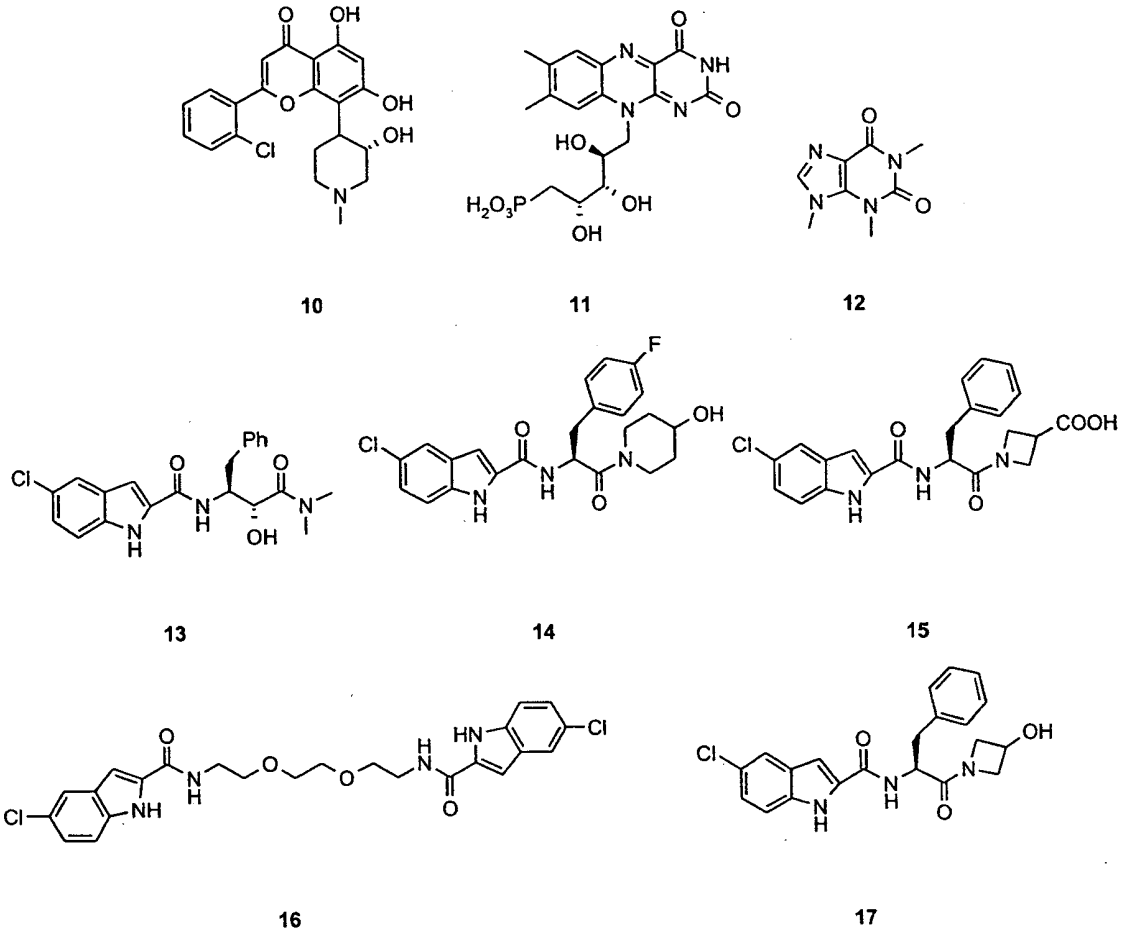
3.2.4 Purine nucleoside site inhibitors

Although this site has not been employed to design inhibitors targeted for therapy, its properties have been extensively studied. Flavopiridol (**10**) and flavin mononucleotide (FMN, **11**) with K_i values of 1 M and 10 M, respectively, against rabbit muscle phosphorylase (*a* or *b*), are the most potent inhibitors shown to bind to this site [59-61]. Caffeine (**12**, K_i = 50 mM) has the highest inhibitory activity of various xanthines that also bind here. These ligands exhibit synergistic inhibition with glucose and appear to derive most of their potency from intercalation of their aromatic rings between the side chains of phenylalanine and tyrosine residues. This intercalation rigidifies the loop of nearby residues which bind glucose at the active site, which in turn accounts for the synergistic inhibition seen when a purine site inhibitor and glucose are both present [62]. The existence of a physiological ligand and its ability to modulate phosphorylase activity through this site has been proposed [63,64].

3.2.5 Indole binding site inhibitors

Compound **13** (CP-91,149) was identified as a 110 nM (IC_{50}) inhibitor of recombinant human liver glycogen phosphorylase *a* (rHLGP*a*), by screening [65]. Subsequent structure-activity studies identified other highly potent rHLGP*a* inhibitors (to 80 nM IC_{50}) in this and an analogous N-(indole-2-carbonyl)-phenylalanine series exemplified by **14** (CP-320,626, IC_{50} 240 nM) [66]. These compounds and caffeine (**12**)

Figure 4: Chemical structures of glycogen phosphorylase inhibitors. *Continued.*



both possess heterocyclic structural elements and show very similar glucose dependence (inhibitor potency increases approximately 5- to 10-fold as glucose concentration is increased from zero to the high end of the physiological range, ca. 10 mM). Nevertheless, a kinetic interaction of **13** with caffeine was seen [65] and subsequently a distinct binding site for these compounds was crystallographically determined. Compound **14** binds to RMGP*b* [67] and compound **15** (CP-403,700, IC_{50} = 45 nM) binds to rHLGP*a* [68,108], in the large symmetric cavity which exists at the subunit interface of the GP homodimer, where no previous ligand interactions are reported (**Figure 3**). One inhibitor is bound in each monomer subunit (two per homodimer). Compound **16** (CP-526,423) has the highest reported enzyme inhibitory activity for rHLGP*a* of any compound (6 nM IC_{50}) that binds to this site. This compound contains two 5-chloroindole-2-carboxamide moieties, joined by a diether linker, which bind identically to those in two molecules of **15** [68].

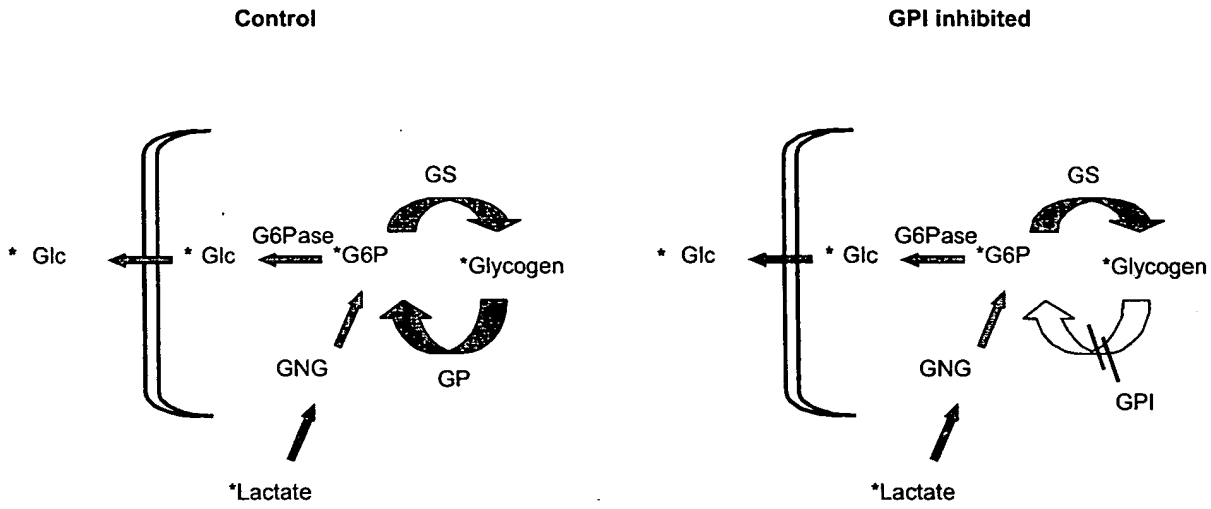
3.3 Pharmacology of glycogen phosphorylase inhibitors (GPIs)

Most of the reports of the pharmacological action of GPIs are recent, consisting of the study of compounds **7** (DAB), **8** (BAYR3401) and indoles **13-14** or analogues, in cell-based and animal models of type 2 diabetes.

3.3.1 Selectivity of phosphorylase inhibitors

Indoles **13** and **14** display similar potencies against human phosphorylase isoforms (liver, brain, muscle) and against non-human phosphorylases (rabbit muscle). This non-selectivity is probably due to the high amino acid sequence identity/homology between these isozymes, particularly at the indole

Figure 5: Mechanism of hepatic glucose-glycogen cycling. The production of glucose (Glc) from glycogenolysis and gluconeogenesis (GNG, from lactate) is shown. Enzymes depicted are glycogen synthase (GS), glycogen phosphorylase (GP) and glucose-6-phosphatase (G6Pase); other enzymes, including phosphoglucomutase which converts glucose-1-phosphate from glycogenolysis to glucose-6-phosphate (G6P), are not shown. The bracket represents the hepatocyte plasma membrane. The scheme summarises the results of radiolabelled studies tracing ^{14}C-lactate (*Lactate) metabolism through the glycogen (*Glycogen) and free glucose (*Glc) pools. In the Control state, *G6P produced from GNG has two fates: 1) direct dephosphorylation by G6Pase to *Glc and 2) cycling through the *Glycogen pool prior to dephosphorylation by G6Pase. In the GPI Inhibited state, GP activity is inhibited and GS is activated, resulting in increased incorporation of radiolabelled carbon from *Lactate into *Glycogen and reduced net *Glc production. The altered flux of GNG-derived G6P in the GPI inhibited state accounts for the apparent inhibition of GNG and the increased glycogenesis produced by CP-91,149 (13) and other indole GPIs.

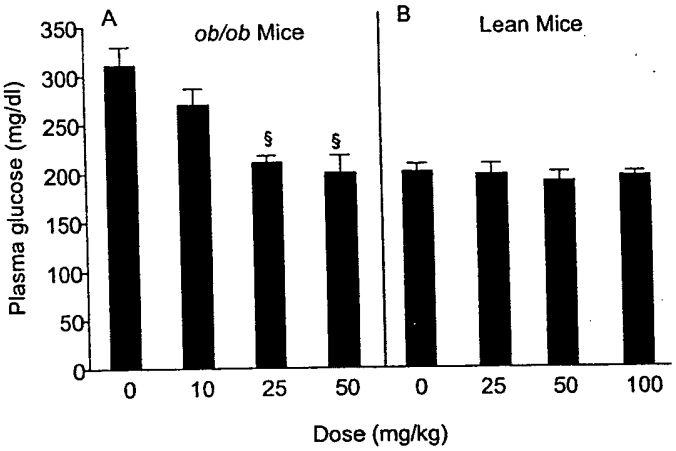


binding site. With respect, however, to interactions with other enzymes, receptors and signalling proteins potentially involved in the regulation of hepatic glucose metabolism, **13** and **14** are very selective, showing no activity at high concentrations against these other targets [65]. Targets examined included -glucosidases, phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, phosphoglucomutase, glucose-6-phosphate dehydrogenase, fructose -1,6-bisphosphatase, aldose reductase, sorbitol dehydrogenase, glycogen synthase, glucagon receptor, glycogen synthase kinase-3 and acetyl CoA carboxylase. Isofagomine (**6**) and DAB (**7**), are reported to inhibit -glucosidases [101,69]. Other data pertaining to the specificity of compounds **6-9** for inhibiting phosphorylase *versus* other targets are not reported.

3.3.2 Inhibition of hepatic glycogenolysis and gluconeogenesis-glycogen cycling

Since hepatic glucose production is the net effect of glycogen breakdown and gluconeogenesis, GPIs were predicted to effect the former but not the latter. This presumes that no interchange of intermediates occurs between the glycogen and gluconeogenic pathways and no hepatic autoregulation, the concept of a compensatory increase in one pathway when the other is inhibited to maintain net hepatic glucose production [24], occurs *in vivo*. To investigate this, inhibitors **13** and **14** were tested for effects on the gluconeogenic rate in hepatocytes and surprisingly a significant decrease was observed [65]. Under these conditions, we observed an expected decrease glycogenolytic activity in the liver cells but ^{14}C-glucose produced from ^{14}C-lactate was markedly reduced, which corresponded with increased ^{14}C-lactate incorporation into hepatic glycogen. Thus, it appears that these glycogen phosphorylase inhibitors indirectly inhibit gluconeogenesis by disrupting the glucose/glycogen cycling involved in hepatic glucose production (**Figure 5**). These results support the contention for significant hepatic glucose-glycogen cycling *in vivo* [38]. A similar finding is suggested for the GPI BAYR3401 [55]. In contrast, studies performed with DAB (**7**) in primary rat hepatocytes demonstrated glycogenolysis inhibition but not glycogen cycling [70]. The other GPIs

Figure 6: Dose-response of glucose-lowering activity by **13** (CP-91,149) in (A) obese, diabetic *ob/ob* mice and (B) lean, non-diabetic control mice. Mice were treated with vehicle or **13** as indicated and plasma glucose concentration was determined 3 h later. Values (mg/dl) represent the mean SEM for 10 - 60 mice per group. Significant ($^{\S}p < 0.01$) decrease by **13** compared with the vehicle control group. Reprinted with permission from [65].



discussed here are not reported to produce a similar effect on gluconeogenesis.

In cell-based assays, GPIs have also been associated with a reduction in the percentage of enzyme present in the active GP*a* form. Both the indole GPIs, as well as D-glucose, 2-deoxyfluoroglucose, N-acetyl--D-glucosamine (**2**), spirohydantoin **3**, BAYR3401 (**8**) and caffeine (**12**), [56,71-74] have been reported to inhibit GP*a* and enhance the dephosphorylation of the GP*a* complex to the inactive conformation GP*b*. This is thought to occur as a result of the binding-induced conformational changes that make GP*a* a better substrate for the regulatory phosphatase PP1 [74]. Indeed, enhanced PP1-mediated dephosphorylation of GP*a* in the presence of GPIs has been demonstrated [68]. Further, by reducing GP*a* levels in cells, PP1 may become disinhibited from the GP*a*-mediated allosteric inhibition that is mediated through the G_L targeting subunit [75]. This could result in an indirect activation of glycogen synthase, which is activated when dephosphorylated by PP1 [56], although a G6P-mediated mechanism is also implicated from some studies using deoxyfluoroglucose analogue inhibitors of GP [72]. Thus, net hepatic glucose production could be reduced under GPI-treated conditions through:

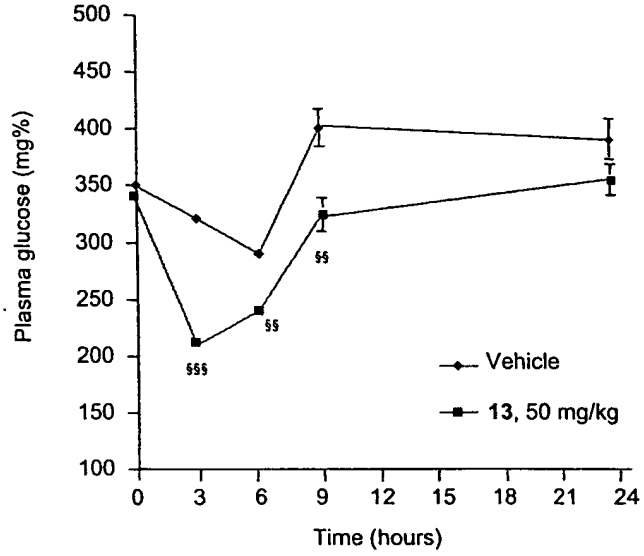
- Direct inhibition of glycogenolysis
- Indirect inhibition of gluconeogenesis
- Indirect activation of glycogen synthase

This hypothesis is consistent with the description of highly regulated liver glycogen metabolism by Bollen [76]. Experiments are ongoing to examine these pathways to better understand their physiological importance toward a GPI's glucose-lowering pharmacology and whether there are any potential limitations, e.g., glycogen storage disease, for this mechanism.

3.3.3 *In vivo* glucose-lowering

GPIs that inhibit liver GP enzymatic activity *in vitro* and reduce glycogenolysis in hepatocytes and/or intact liver also display acute glucose-lowering *in vivo* in diabetic *ob/ob* mice. The GPIs **13** and **14** have demonstrated dose-dependent glucose-lowering at 3 h following a single oral dose (**Figures 6** and **7**) [65,66]. For **14**, effective doses result in glucose-lowering for up to 10 h post-dose; by 24 h post-dose, the **14**-treated animals returned to their hyperglycaemic baseline and are indistiguishable from vehicle-treated controls. The time course for glucose-lowering efficacy by **14** is consistent with its pharmacokinetic properties (plasma and liver half-life) in the diabetic *ob/ob* mouse. The glucose-lowering observed by GPI treatment is associated with the acute inhibition of hepatic glycogenolysis *in vivo* as determined using a radiolabelling technique [65]. Several other glycogen phosphorylase inhibitors, specifically compounds **7** (DAB), [54] **8** (BAYR3401), [55] and 2,5-anhydro-D-mannitol [78] have also demonstrated acute glucose-lowering in diabetes-related models *in vivo*. Cumulatively, these results

Figure 7: Time course of glucose-lowering following oral dosing of 13 in diabetic *ob/ob* mice. Vertical lines show SEM. Each point represents 35 - 66 mice per group. Significance ($^{§§§}p < 0.001$, $^{§§}p < 0.01$) by unpaired t-test relative to vehicle control groups. Data reprinted with permission from [66].



support the contention that hepatic glycogenolysis is an important contributor to diabetes-associated hyperglycaemia and that inhibition of hepatic glycogen phosphorylase results in significant glucose-lowering *in vivo*.

3.3.4 Multiple dose effects

In preliminary studies, we administered compound **14** to diabetic *ob/ob* mice for two weeks to determine multiple dose glucose-lowering efficacy, tissue glycogen levels and effects on restoration of metabolic control [78]. Chronic administration of **14** (10 mg/kg, b.i.d.) was associated with sustained reductions in fed-state glucose levels over the two-week study period. Fasting glucose levels were unaffected by **14**, consistent with a reduced hepatic glycogen content fasted state. Oral glucose tolerance was improved by treatment with **14**, as reflected in reduced peak glucose excursion during the OGTT. Modest (+ 30%) elevation of hepatic glycogen content was observed five days after the initiation of **14** treatment but further elevation despite continued dosing was not observed between days 5 and 15 of the study. Likewise, treatment with **14** was associated with increased muscle (3- to 6-fold) glycogen concentration that reached a plateau by day 5 of the study. Importantly, mobilisation of skeletal muscle glycogen during electrically induced leg muscle contractions was not impaired by chronic **14** treatment in these diabetic *ob/ob* mice. Further, chronic administration of **14** was associated with reduced plasma lipids and lactate levels and was without compensatory hyperglucagonaemia. Collectively, the results suggest that GPIs could be useful as chronic therapy for controlling hyperglycaemia and possibly restoring secondary metabolic control in type 2 diabetics.

3.3.5 Lack of hypoglycaemia

Indole GPIs have been studied in a variety of preclinical animal models and treatment has not been associated with incidence of hypoglycaemia. In diabetic *ob/ob* mice, glucose-lowering is observed to the limit of normoglycaemia on treatment with compounds **13** or **14** at doses up to 50 mg/kg and in non-diabetic littermates, mice remain euglycaemic to doses as high as 100 mg/kg [65,66,78]. We hypothesise that the *in vitro* glucose dependence displayed by GPIs for inhibiting the GP enzyme affords *in vivo* efficacy predominantly in hyperglycaemic states, which is an important safety consideration in developing new therapeutic agents for treating diabetes.

3.3.6 Potential for cardioprotective effects

In evaluating liver glycogen phosphorylase inhibitors for potential use as antidiabetic agents, compounds were identified that also inhibited the muscle and brain phosphorylase isoforms. Since both muscle and brain glycogen phosphorylases are expressed in the

myocardium and glycogen breakdown during ischaemia/reperfusion injury may contribute to infarct development [79], we examined whether a indole GPI could provide cardioprotection. A rabbit Langendorff model of ischaemia/reperfusion injury was utilised to evaluate the cardioprotective effects of constant perfusion with 5 M of compound 17, an inhibitor of the human brain (IC_{50} = 55 nM) and human muscle (IC_{50} = 121 nM) GP isoforms. Examination of the cardiac tissue at the end of the ischaemic/reperfusion period showed a 50% reduction ($p < 0.05$) in the infarct area relative to the area at risk due to CP-380867 treatment, compared with untreated hearts [80,109]. Since glycogen breakdown and anaerobic metabolism of the glucose produced can lead to the production of lactate and [H^+] ions which are thought to contribute to the myocardial damage incurred during ischaemia [81], the GPI's apparent benefit is to reduce the production of these metabolites from glycogen. Recently, similar cardioprotective findings have been reported for inhibitors of the glycogen debranching enzyme, MOR-14 and miglitol [82]. These results indicate that agents that lower glucose by inhibiting glycogenolysis may also provide protection to the myocardium during periods of ischaemic injury. As type 2 diabetic patients are at increased risk for the development of cardiomyopathies and display increased morbidity and mortality due to cardiovascular diseases, an agent that both lowers glucose and positively impacts myocardial metabolism may be advantageous as a therapeutic agent.

4. Expert opinion and future directions

Preclinical evidence strongly suggests that inhibition of hepatic glycogen phosphorylase could be an effective approach to achieve glucose-lowering in patients with type 2 diabetes. The indole GPIs discovered at Pfizer Global Research & Development are believed to be the first and most advanced agents developed toward this end. Compounds in this class have been studied clinically, currently to Phase II and preliminary results have confirmed their glucose-lowering potential. Based on a novel mechanism of action by inhibition of a specific molecular target, hepatic glycogen phosphorylase, we are optimistic that clinical agents will be successfully developed to lower glucose without hypoglycaemic risk, as monotherapy or in combination with other agents, based on complementary mechanisms of action. Furthermore, other distinctive features of a GPI, such as cardioprotection, may impact the cardiovascular risk in the diabetic population and serve to differentiate a GPI as an agent of choice for drug therapy.

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Bibliography

Papers of special note have been highlighted as:
- • of interest
- •• of considerable interest

1. KEEN H, CLARK C, LAAKSO M: **Reducing the burden of diabetes: managing cardiovascular disease.** *Diabetes Metab. Res. Rev.* (1999) 15:186-196.
   - •• Insightful comments on the relationship between diabetes and macrovascular complications/cardiovascular disease. It supports the current recommendations for optimising care of patients with diabetes who are at significant risk for CHD.
2. LIM SC, TAI ES, TAN BY, CHEW SK, TAN CE: **Cardiovascular risk profile in individuals with borderline glycemia: the effect of the 1997 American Diabetes Association diagnostic criteria and the 1998 World Health Organization Provisional Report.** *Diabetes Care* (2000) 23:278-282.
3. STERN MP: **The effect of glycemic control on the incidence of macrovascular complications of Type 2 diabetes.** *Arch. Fam. Med.* (1998) 7:155-162.
   - • Practical commentary on identifying the benefits of aggressive glycaemic control as related to macrovascular disease.
4. WILD SH, DUNN CJ, MCKEIGUE PM, COMTE S: **Glycemic control and cardiovascular disease in type 2 diabetes: a review.** *Diabetes Metab. Res. Rev.* (1999) 15:197-204.
   - • A useful summary to position the importance of glycaemic control relative to general risk reduction approaches in the management of type 2 diabetes.
5. CLARK CM, FRADKIN JE, HISS RG, LORENZ RA, VINICOR F, WARREN-BOULTON E: **Promoting early diagnosis and treatment of type 2 diabetes: the National Diabetes Education Program.** *JAMA* (2000) 284:363-365.
   - • An excellent position paper on how the healthcare system should view the evolving epidemic of diabetes and prepare to make adaptations in meeting the public health demands of the disease from the patient and clinician perspectives.
6. **American Diabetes Association. Economic consequences of diabetes mellitus in the US in 1997.** *Diabetes Care* (1998) 21:296-309.

7. DEFRONZO RA: **Pharmacologic therapy for type 2 diabetes mellitus.** *Ann. Intern. Med.* (1999) 131:281-303.
•• A contemporary review of the pathophysiology and medical treatment strategy for type 2 diabetes mellitus by a diabetes leader.

8. NATIONAL DIABETES DATA GROUP: **Diabetes In America (2nd edition).** National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, NIH Publication No. 95-1468 (1995).

9. US DEPARTMENT OF HEALTH AND HUMAN SERVICES CFDCAP: **'National Diabetes Fact Sheet'.** 1st November (1998):1-8.

10. HARRIS MI, FLEGAL KM, COWIE CC *et al.*: **Prevalence of diabetes, impaired fasting glucose and impaired glucose tolerance in US adults. The Third National Health and Nutrition Examination Survey, 1988-1994.** *Diabetes Care* (1998) 21:518-524.

11. KING H, AUBERT RE, HERMAN WH: **Global burden of diabetes, 1995-2025: prevalence, numerical estimates and projections.** *Diabetes Care* (1998) 21:1414-1431.

12. KOPELMAN PG, HITMAN GA: **Diabetes. Exploding Type II.** *Lancet* (1998) 352:SIV5.

13. AMOS AF, MCCARTY DJ, ZIMMET P: **The rising global burden of diabetes and its complications: estimates and projections by 2010.** *Diab. Med.* (1997) 14(Suppl. 5):S5-S85.

14. HAFFNER SM, LEHTO S, RONNEMAA T, PYORALA K, LAAKSO M: **Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction.** *N. Engl. J. Med.* (1998) 339:229-234.
•• This paper provides extremely important data on how we should position the issue of cardiovascular risk in patients with type 2 diabetes and brings to light a perspective that is useful both clinically and from a policy point of view.

15. AMERICAN DIABETES ASSOCIATION: **Implications of the United Kingdom Prospective Diabetes Study.** *Diabetes Care* (2001) 24:S28-S32.
•• Useful summary and updated reference on how the findings from the UKPDS can be interpreted for clinical decision-making.

16. FLEMING A: **FDA approach to the regulation of drugs for diabetes.** *Am. Heart. J.* (1999) 138:S338-S345.

17. PSATY BM, WEISS NS, FURBERG CD *et al.*: **Surrogate end points, health outcomes and the drug-approval process for the treatment of risk factors for cardiovascular disease.** *JAMA* (1999) 282:786-790.

18. DEFRONZO RA, BONADONNA RC, FERRANNINI E: **Pathogenesis of NIDDM. A balanced overview.** *Diabetes Care* (1992) 15:318-368.
• A useful summary of the pathophysiology of type 2 diabetes.

19. WITHERS DJ, WHITE M: **Perspective: the insulin signaling system - a common link in the pathogenesis of type 2 diabetes.** *Endocrinology* (2000) 141:1917-1921.
• Brings new observations on insulin signalling to the forefront of understanding the various pathogenesis of diabetic hyperglycaemia and insulin resistance.

20. CONSOLI A: **Role of liver in pathophysiology of NIDDM.** *Diabetes Care* (1992) 15:430-441.
• Though nearly a decade old, this paper classically delineates hepatic dysfunction contributing to diabetic hyperglycaemia.

21. EDELMAN SV: **Type II diabetes mellitus.** *Adv. Intern. Med.* (1998) 43:449-500.

22. VAAG A: **On the pathophysiology of late onset non-insulin dependent diabetes mellitus. Current controversies and new insights.** *Dan. Med. Bull.* (1999) 46:197-234.
• A comprehensive current review of type 2 diabetes mellitus with 554 references.

23. LEFEBVRE PJ, SCHEEN AJ: **Glucose metabolism and the postprandial state.** *Eur. J. Clin. Invest.* (1999) 29:1-6.

24. TAPPY L: **Regulation of hepatic glucose production in healthy subjects and patients with non-insulin-dependent diabetes mellitus.** *Diabetes Metab.* (1995) 21:233-240.

25. RIZZA R: **The role of splanchnic glucose appearance in determining carbohydrate tolerance.** *Diabetes Med.* (1996) 13:S23-S27.

26. EKBERG K, LANDAU BR, WAJNGOT A *et al.*: **Contributions by kidney and liver to glucose production in the postabsorptive state and after 60 h of fasting.** *Diabetes* (1999) 48:292-298.

27. PREVIS SF, CLINE GW, SHULMAN GI: **A critical evaluation of mass isotopomer distribution analysis of gluconeogenesis *in vivo*.** *Am. J. Physiol.* (1999) 277:E154-E160.

28. PREVIS SF, HALLOWELL PT, NEIMANIS KD, DAVID F, BRUNENGRABER H: **Limitations of the mass isotopomer distribution analysis of glucose to study gluconeogenesis. Heterogeneity of glucose labeling in incubated hepatocytes.** *J. Biol. Chem.* (1998) 273:16853-16859.

29. ADZIUK J, LEE WP: **Measurement of gluconeogenesis and mass isotopomer analysis based on [U-(13)C]glucose.** *Am. J. Physiol.* (1999) 277:E199-E207.
• A critical review of the theory and practice of mass isotopomer determination of gluconeogenesis *in vivo*.

30. ELLEHER JK: **Estimating gluconeogenesis with [U-13C]glucose: molecular condensation requires a molecular approach.** *Am. J. Physiol.* (1999) 277:E395-E400.

31. RODEN M: **NMR and stable isotope techniques for studies of liver metabolism in man.** *Horm. Metab. Res.* (1997) 29:340-343.
• A concise review of *in vivo* ^{13}C NMR spectroscopy-based hepatic glycogen metabolism techniques.

32. BLOCH G, VELHO G: **Metabolic investigations in humans by *in vivo* nuclear magnetic resonance. Recommendations of ALFEDIAM (French Language Association for the Study of Diabetes and Metabolic Diseases).** *Diabetes. Metab.* (1997) 23:343-350.

33. BARRETT EJ, LIU Z: **Hepatic glucose metabolism and insulin resistance in NIDDM and obesity.** *Baillieres Clin. Endocrinol. Metab.* (1993) 7:875-901.
•• Excellent review of the literature through 1992 on the role of glycogenolysis in hepatic glucose output in diabetes.

34. GASTALDELLI A, BALDI S, PETTITI M *et al.*: **Influence of obesity and type 2 diabetes on gluconeogenesis and glucose output in humans: a quantitative study.** *Diabetes* (2000) 49:1367-1373.

35. LANDAU BR, WAHREN J, CHANDRAMOULI V, SCHUMANN WC, EKBERG K, KALHAN SC: **Contributions of gluconeogenesis to glucose production in the fasted state.** *J. Clin. Invest.* (1996) 98:378-385.

36. ROTHMAN DL, MAGNUSSON I, KATZ LD, SHULMAN RG, SHULMAN GI: **Quantitation of hepatic glycogenolysis and gluconeogenesis in fasting humans with ^{13}C NMR.** *Science* (1991) 254:573-576.

37. MAGNUSSON I, ROTHMAN DL, KATZ LD, SHULMAN RG, SHULMAN GI: **Increased rate of gluconeogenesis in Type II diabetes mellitus. A ^{13}C nuclear magnetic resonance study.** *J. Clin. Invest.* (1992) 90:1323-1327.

38. HELLERSTEIN MK, NEESE RA, LINFOOT P, CHRISTIANSEN M, TURNER S, LETSCHER A: **Hepatic gluconeogenic fluxes and glycogen turnover during fasting in humans. A stable isotope study.** *J. Clin. Invest.* (1997) 100:1305-1319.

39. PIMENTA W, NURJHAN N, JANSSON PA, STUMVOLL, M, GERICH J, KORYTKOWSKI M: **Glycogen: its mode of formation and contribution to hepatic glucose output in postabsorptive humans.** *Diabetologia* (1994) 37:697-702.

40. TAYEK JA, KATZ J: **Glucose production, recycling and gluconeogenesis in normals and diabetics: a mass isotopomer [U-13C]glucose study.** *Am. J. Physiol.* (1996) 270:E709-E717.

41. DIRAISON F, LARGE V, BRUNENGRABER H, BEYLOT M: **Non-invasive tracing of liver intermediary metabolism in normal subjects and in moderately hyperglycaemic NIDDM subjects. Evidence against increased gluconeogenesis and hepatic fatty acid oxidation in NIDDM.** *Diabetologia* (1998) 41:212-220.
• Stable isotope analysis study indicating that gluconeogenesis is not the major contributor to hepatic glucose production in patients with type 2 diabetes mellitus.

42. VAN DEN BERGHE G: **The role of the liver in metabolic homeostasis: implications for inborn errors of metabolism.** *J. Inherit. Metab. Dis.* (1991) 14:407-420.

43. NEWGARD CB, HWANG PK, FLETTERICK RJ: **The family of glycogen phosphorylases: structure and function.** *Crit. Rev. Biochem. Mol. Biol.* (1989) 24:69-99.
•• A comprehensive review with 158 references of phosphorylase structure and function from the authors who first cloned human liver glycogen phosphorylase.

44. SPRANG SR, GOLDSMITH EJ, FLETTERICK RJ, WITHERS SG, MADSEN NB: **Catalytic site of glycogen phosphorylase: structure of the T state and specificity for α-D-glucose.** *Biochemistry* (1982) 21:5364-5371.

45. MARTIN JL, VELURAJA K, ROSS K *et al.*: **Glucose analog inhibitors of glycogen phosphorylase: the design of potential drugs for diabetes.** *Biochemistry* (1991) 30:10101-10116.

46. WATSON KA, MITCHELL EP, JOHNSON LN *et al.*: **Design of Inhibitors of Glycogen Phosphorylase: A Study of α- and β-C-Glucosides and 1-Thio-β-D-glucose Compounds.** *Biochemistry* (1994) 33:5745-5758.
•• A key reference on the design, synthesis, structure-activity relationships and crystallographically-determined binding of glucose analogue inhibitors.

47. WATSON KA, MITCHELL EP, JOHNSON LN: **Glucose analog inhibitors of glycogen phosphorylase: from crystallographic analysis to drug prediction using GRID force-field and GOLPE variable selection.** *Acta. Crystallogr. Sect. D: Biol. Crystallogr.* (1995) D51:458-472.

48. GREGORIOU M, NOBLE MEM, WATSON KA *et al.*: **The structure of a glycogen phosphorylase glucopyranose spirohydantoin complex at 1.8 ANG. resolution and 100 K: the role of the water structure and its contribution to binding.** *Protein Sci.* (1998) 7:915-927.

49. OSZ E, SOMSAK L, SZILAGYI L *et al.*: **Efficient inhibition of muscle and liver glycogen phosphorylases by a new glucopyranosylidene-spirothiohydantoin.** *Bioorg. Med. Chem. Lett.* (1999) 9:1385-1390.

50. MITCHELL EP, WITHERS SG, ERMERT P *et al.*: **Ternary complex crystal structures of glycogen phosphorylase with the transition state analog nojirimycin tetrazole and phosphate in the T and R states.** *Biochemistry* (1996) 35:7341-7355.

51. ASANO N, NASH RJ, MOLYNEUX RJ, FLEET GWJ: **Sugar-mimic glycosidase inhibitors: natural occurrence, biological activity and prospects for therapeutic application.** *Tetrahedron: Asymmetry* (2000) 11:1645-1680.

52. WAAGEPETERSEN HS, WESTERGAARD N, SCHOUSBOE A: **The effects of isofagomine, a potent glycogen phosphorylase inhibitor, on glycogen metabolism in cultured mouse cortical astrocytes.** *Neurochem. Int.* (2000) 36:435-440.

53. LUNDGREN K, RASSOV A, BOLS M: **NN42-1007 is a novel potent inhibitor of hepatic glycogen phosphorylase and of hepatocyte glycogenolysis.** *Diabetes* (1996) 45:521.

54. FOSGERAU K, WESTERGAARD N, QUISTORFF B, GRUNNET N, KRISTIANSEN, M, LUNDGREN, K: **Kinetic and functional characterization of 1,4-dideoxy-1,4-imino-d-arabinitol: a potent inhibitor of glycogen phosphorylase with anti-hyperglycaemic effect in ob/ob mice.** *Arch. Biochem. Biophys.* (2000) 380:274-284.
•• Demonstration of antidiabetic potential in a non-indole glycogen phosphorylase inhibitor.

55. SHIOTA M, JACKSON PA, BISCHOFF H *et al.*: **Inhibition of glycogenolysis enhances gluconeogenic precursor uptake by the liver of conscious dogs.** *Am. J. Physiol.* (1997) 273:E868-E879.
•• First demonstration of quantitative importance of glycogenolysis to hepatic glucose production in a non-rodent efficacy model.

56. BERGANS N, STALMANS W, GOLDMANN S, VANSTAPEL F: **Molecular mode of inhibition of glycogenolysis in rat liver by the dihydropyridine derivative, BAY R3401; Inhibition and inactivation of glycogen phosphorylase by an activated metabolite.** *Diabetes* (2000) 49:1419-1426.
• Elegant detailed demonstration of the inter-regulation of glycogen synthesis and degradation as studied with the AMP-site GPI BAY R3401.

57. ZOGRAPHOS SE, OIKONOMAKOS NG, TSITSANOU KE *et al.*: **The structure of glycogen phosphorylase *b* with an alkyl-dihydropyridine-dicarboxylic acid compound, a novel and potent inhibitor.** *Structure* (1997) 5:1413-1425.

58. OIKONOMAKOS NG, TSITSANOU KE, ZOGRAPHOS SE, SKAMNAKI VT, GOLDMANN S, BISCHOFF H: **Allosteric inhibition of glycogen phosphorylase *a* by the potential antidiabetic drug 3-isopropyl 4-(2-chlorophenyl)-1,4-dihydro-1-ethyl-2-methyl-pyridine-3,5,6-tricarboxylate.** *Protein. Sci.* (1999) 8:1930-1945.

59. OIKONOMAKOS NG, SCHNIER JB, ZOGRAPHOS SE, SKAMNAKI VT, TSITSANOU, KE, JOHNSON LN: **Flavopiridol inhibits glycogen phosphorylase by binding at the inhibitor site.** *J. Biol. Chem.* (2000) 275:34566-34573.
• Recent discussion of enzyme-inhibitor interactions at the purine nucleoside inhibitor (caffeine) binding site with leading references.

60. SPRANG S, FLETTERICK R, STERN M, YANG D, MADSEN N, STURTEVANT J: **Analysis of an allosteric binding site: the nucleoside inhibitor site of phosphorylase alpha.** *Biochemistry* (1982) 21:2036-2048.
• Seminal description of the purine nucleoside inhibitor (caffeine) binding site.

61. KLINOV SV, KURGANOV BI: **Specificity of inhibition of muscle glycogen phosphorylase β by flavins.** *Biochem. Mol. Biol. Int.* (1995) 35:643-650.

62. KASVINSKY PJ, SHECHOSKY S, FLETTERICK RJ: **Synergistic regulation of phosphorylase *a* by glucose and caffeine.** *J. Biol. Chem.* (1978) 253:9102-9106.
• Determination of the basis of synergy between catalytic and purine nucleoside inhibitor site ligands from kinetic data of rabbit liver and muscle phosphorylase with reference to the crystal structure of the rabbit muscle enzyme.

63. ERCAN-FANG N, NUTTALL FQ: **The effect of caffeine and caffeine analogs on rat liver phosphorylase *a* activity.** *J. Pharmacol. Exp. Ther.* (1997) 280:1312-1318.

64. ERCAN-FANG, NG, NUTTALL FQ, GANNON MC: **Uric acid inhibits liver phosphorylase *a* activity under simulated *in vivo* conditions.** *J. Physiol. Endocrinol. Metab.* (2001) 280:E248-E253.

65. MARTIN WH, HOOVER DJ, ARMENTO SJ *et al.*: **Discovery of a human liver glycogen phosphorylase inhibitor that lowers blood glucose *in vivo*.** *Proc. Natl. Acad. Sci. USA.* (1998) 95:1776-1781.
•• Identification of the glycogen phosphorylase inhibitory activity of compound 13 (CP-91,149) including characterisation of acute hypoglycaemic activity in the *ob/ob* mouse.

66. HOOVER DJ, LEFKOWITZ-SNOW S, BURGESS-HENRY JL *et al.*: **Indole-2-carboxamide inhibitors of human liver glycogen phosphorylase.** *J. Med. Chem.* (1998) 41:2934-2938.
•• Description of the chemical synthesis, structure-activity relationships and biological characterisation of analogues of compounds 13 (CP-91,149) and 14 (CP-320,626).

67. OIKONOMAKOS NG, SKAMNAKI VT, TSITSANOU KE, GAVALAS NG, JOHNSON LN: **A new allosteric site in glycogen phosphorylase b as a target for drug interactions.** *Structure (London)* (2000) 8:575-584.
• Characterisation of the binding site of compound 14 (CP-320,626) in rabbit muscle glycogen phosphorylase *b*.

68. RATH VL, AMMIRATI M, DANLEY DE *et al.*: **Human liver glycogen phosphorylase inhibitors bind at a new allosteric site.** *Chem. Biol.* (2000) 7:677-682.
•• Characterisation of the indole inhibitor binding site (*via* compound 15) in recombinant human liver glycogen phosphorylase a and design of a novel inhibitor (16) from this x-ray data.

69. DONG W, JESPERSEN T, BOLS M, SKRYDSTRUP T, SIERKS MR: **Evaluation of Isofagomine and Its Derivatives As Potent Glycosidase Inhibitors.** *Biochemistry* (1996) 35:2788-2795.

70. ANDERSEN B, RASSOV A, WESTERGAARD N, LUNDGREN K: **Inhibition of glycogenolysis in primary rat hepatocytes by 1,4-dideoxy-1,4-imino-D-arabinitol.** *Biochem. J.* (1999) 342:545-550.

71. BOARD M, BOLLEN M, STALMANS W, YONG K, FLEET GWJ, JOHNSON LN: **Effects of C-1-substituted glucose analog on the activation states of glycogen synthase and glycogen phosphorylase in rat hepatocytes.** *Biochem. J.* (1995) 311:845-852.

72. MASSILLON D, BOLLEN M, DE WULF H *et al.*: **Demonstration of a glycogen/glucose 1-phosphate cycle in hepatocytes from fasted rats. Selective inactivation of phosphorylase by 2-deoxy-2-fluoro-alpha-D-glucopyranosyl fluoride.** *J. Biol. Chem.* (1995) 270:19351-19356.

73. SOMSAK L, NAGY V, DOCSA T, TOTH B, GERGELY P: **Gram-scale synthesis of a glucopyranosylidene-spirothiohydantoin and its effect on hepatic glycogen metabolism studied *in vitro* and *in vivo*.** *Tetrahedron: Asymmetry* (2000) 11:405-408.

74. KASVINSKY PJ, FLETTERICK RJ, MADSEN NB: **Regulation of the dephosphorylation of glycogen phosphorylase a and synthase b by glucose and caffeine in isolated hepatocytes.** *Can. J. Biochem.* (1981) 59:387-395.

75. ARMSTRONG CG, DOHERTY MJ, COHEN PT: **Identification of the separate domains in the hepatic glycogen-targeting subunit of protein phosphatase 1 that**

interact with phosphorylase *a*, glycogen and protein phosphatase 1. *Biochem. J.* (1998) **336**:699-704.
• Informative paper linking the allosteric inhibition of protein phosphatase 1 by glycogen phosphorylase *a* to specific binding with the glycogen targeting subunit.

76. BOLLEN M, KEPPENS S, STALMANS W: **Specific features of glycogen metabolism in the liver.** *Biochem. J.* (1998) **336**:19-31.
•• An excellent current review on the biochemical regulation of hepatic glycogen metabolism.

77. HANSON RL, HO RS, WISEBERG JJ, SIMPSON R, YOUNATHAN ES, BLAIR JB: **Inhibition of gluconeogenesis and glycogenolysis by 2,5-anhydro-D-mannitol.** *J. Biol. Chem.* (1984) **259**:218-223.

78. TREADWAY JL, MCPHERSON RK, GENEREUX PE *et al.*: **The human liver glycogen phosphorylase inhibitor CP-320,626 shows sustained glucose lowering on multiple dosing in diabetic ob/ob mice.** *Diabetes* (1998) **47**(Suppl.1):1115.
• Demonstration of the multiple dose antidiabetic efficacy of compound **14** (CP-320626) in a preclinical model of human diabetes.

79. KING LM, OPIE LH: **Glucose and glycogen utilisation in myocardial ischemia - changes in metabolism and consequences for the myocyte.** *Mol. Cell.Biochem.* (1998) **180**:3-26.
• A good review of the relationship between carbohydrate metabolism and cardiac function in normal and pathophysiological states.

80. TREADWAY JL, MAGEE WP, HOOVER DJ *et al.*: **Cardioprotective effect of the glycogen phosphorylase inhibitor CP-380867.** *Diabetes* (2000) **49**(Suppl. 1):517

81. LAVANCHY N, GRABLY S, GARNIER A, ROSSI A: **Crucial role of intracellular effectors on glycogenolysis in the isolated rat heart: potential consequences on the myocardial tolerance to ischemia.** *Mol. Cell. Biochem.* (1996) **160-161**:273-282.

82. MINATOGUCHI S, ARAI M, UNO Y *et al.*: **A novel anti-diabetic drug, miglitol, markedly reduces myocardial infarct size in rabbits.** *Br. J. Pharmacol.* (1999) **128**:1667-1672.
• This elegant study demonstrates that the glycogen debranching enzyme inhibitor miglitol provides cardioprotection *in vivo*.

83. CARSON M: **Ribbons 2.0.** *J. Appl. Crystallog.* (1991) **24**:958-961.

84. RATH VL, AMMIRATI M, LEMOTTE PK *et al.*: **Activation of human liver glycogen phosphorylase by alteration of the secondary structure and packing of the catalytic core.** *Mol. Cell* (2000) **6**:139-148.

Patents

101. NOVO NORDISK AV: WO9709040A1 (1997).
102. NOVO NORDISK AV: WO9524391A1 (1995).
103. NOVO NORDISK AV: WO9850359A1 (1998).
104. NOVO NORDISK: US5863903 (1999).
105. BAYER AKTIENGESELLSCHAFT: US5026714 (1991).
106. BAYER AKTIENGESELLSCHAFT: US4894378 (1990).
107. BAYER AKTIENGESELLSCHAFT: US4786641 (1988).
108. PFIZER PRODUCTS INC: EP978279A1 (February 9, 2000).
109. PFIZER INC: US595322 (1999).

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